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Deuel, J W ; Lutz, H U ; Misselwitz, B ; Goede, J S

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Asymptomatic elevation of the hyperchromic red blood cell subpopulation is associated with decreased red cell deformability

Jeremy W. Deuel · Hans U. Lutz · Benjamin Misselwitz · Jeroen S. Goede

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Abstract Hyperchromasia of the red blood cells (RBC), defined as an elevation of the hyperchromic subpopulation, has been described for various medical conditions. However, neither the association of hyperchromasia with an altered RBC membrane nor with other medical conditions has been investigated in a systematic way so far. Since the percentage of hyperchromic RBC is measured on a routine basis by many hematologic laboratories, we evaluated the predictive value of this parameter for the detection of RBC disorders. An extensive workup of all patients undergoing standard hematogram during a period of 6 months at our institution with a fraction of hyperchromic RBC larger than 10 % was collected by reviewing the medical history and performing osmotic gradient ektacytometry on RBC from a part of these patients. Thirty-two thousand two hundred twenty-six individuals were screened; of which, 162 (0.5 %) showed more than 10 % hyperchromic RBC. All of the patients examined by ektacytometry featured abnormal membrane deformability. Hereditary spherocytosis was found in 19 out of these 32 patients, in most cases unknown to the patient and currently asymptomatic. Another 17.9 % of the patients with an elevated subpopulation of hyperchromic RBC suffered from viral

infection (human immunodeficiency virus, hepatitis). Our study shows that an elevated proportion of hyperchromic erythrocytes larger than 10 % is associated with both hereditary and acquired RBC membrane disorders and further follow-up should be considered.

Keywords Hyperchromasia · Spherocytosis · Red blood cells · Membrane

Introduction

Hyperchromasia of the red blood cells (RBC) is a common feature of various conditions and has been described especially for hereditary spherocytosis (HS) [1] but also for various others, such as listed in Table 1 [2–4]. Hyperchromasia, defined as an elevated percentage of hyperchromic RBC, can be predictive for an altered RBC membrane even in the absence of an elevated mean cellular hemoglobin concentration (MCHC) [5].

HS is a common form of hereditary RBC membranopathies. Symptoms range from asymptomatic forms to occasional severe diseases requiring splenectomy and regular transfusions; a large portion of patients are asymptomatic and unaware of their condition [6]. In general practice, HS is typically diagnosed by osmotic fragility testing [7, 8]; however, mild forms of hereditary RBC membranopathies with only limited hemolytic activity are often difficult to identify [9]. In these cases, osmotic fragility tests have a poor sensitivity and at least 20 % of mild cases of HS are missed [3]. In contrast to this, osmotic gradient ektacytometry with the simultaneous recording of deformability against the solute concentration remains a gold standard for the diagnosis of altered erythrocyte deformability [6, 10]. However, this assay is technically challenging, requiring cumbersome calibration

J. W. Deuel · J. S. Goede (✉)
Division of Hematology, University Hospital of Zurich,
Rämistrasse 100,
8091 Zurich, Switzerland
e-mail: jeroen.goede@usz.ch

H. U. Lutz
Institute of Biochemistry, ETH Zurich,
Zurich, Switzerland

B. Misselwitz
Division of Gastroenterology, University Hospital of Zurich,
Zurich, Switzerland

Table 1 Known conditions causing hyperchromasia of RBC

Hereditary RBC membranopathies such as HS
AIHA
Microangiopathic and macroangiopathic hemolytic anemias
HELLP syndrome
Hemolytic transfusion reactions
Cytotoxic treatment
Artificial heart valves
Massive myocardial infarction
Acute oxidant injury
Thermal injuries
Liver disease
Clostridial sepsis
Zinc toxicity
Poisoning by certain snake, spider, and hymenoptera venoms
Severe hypophosphatemia
Hypersplenism
Severe hypoosmolality of the plasma ^a

^a Pseudohyperchromasia due to an artifact of the automatic determination of the RBC indices

and freshly obtained blood, and is, therefore, not suitable as a screening test in clinical routine.

Regarding the existing literature, it has to be noted that the significance of hyperchromasia as an isolated laboratory parameter is unclear and its clinical interpretation poses a major challenge. However, neither the association of hyperchromasia with an altered RBC membrane nor with other medical conditions, such as viral infection, has been investigated in a systematic way until now. The percentage of hyperchromic RBC has been used before as a screening tool for RBC membranopathies [11]; however, no rigorous follow-up of patients was attempted and the diagnostic significance of an elevated percentage of hyperchromic RBC remains unclear.

In this paper, we present a systematic study of patients showing a marked elevation of the hyperchromic RBC subpopulation. To detect altered RBC membrane deformability at a high level of sensitivity, we used osmotic gradient ektacytometry. By reviewing the medical history of hyperchromic patients, we were also able to detect an association with other pathological conditions such as viral infections.

Design and methods

Comparing the percentage of hyperchromic RBCs with MCHC

To evaluate the distribution of the percentage of hyperchromic RBCs and MCHC in our patient population, we assessed all patients undergoing standard hematogram at the central hematologic laboratory in the University

Hospital of Zurich within a period of 2 months (Fig. 1). If samples of a patient were measured on several occasions, only the sample with the highest percentage of hyperchromic RBC was considered. All hematograms were analyzed by an ADVIA 120 (Siemens, Forchheim, Germany). Erythrocytes with a corpuscular hemoglobin concentration of more than 410 g/l were considered hyperchromic, whereas the upper limit for the mean corpuscular hemoglobin concentration (MCHC) is defined as 360 g/l. To minimize measurement errors, all samples with an obvious pathological deviation of a measurement on hematological parameters were measured at least twice.

All hematological parameters measured by the ADVIA system, including the hyperchromic RBC fraction, were calibrated and quality controlled on a regular basis, complying with good laboratory practice. The linear range of single cell hemoglobin concentration measured by the ADVIA system ranges from 0 to 500 g/l. Statistical analysis was done using Graphpad Prism.

Patient recruitment and data collection

From all patients undergoing a standard hematogram during a period of 6 months (February until August 2008), we selected all individuals with 10 % or more hyperchromic erythrocytes. This cutoff value was arbitrarily chosen to include approximately 0.3 % of all patients. The medical

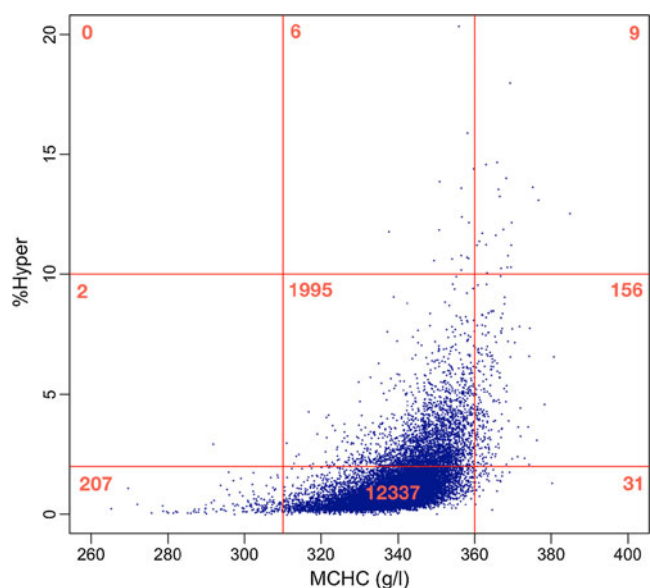


Fig. 1 Plot of MCHC as a function of the fraction of hyperchromic RBC. Fourteen thousand nine hundred eighty-eight measurements of patients at our institution during a period of 2 months are shown. The vertical lines indicate the upper and lower limits of normal (>360 and <310 g/l, respectively). Similarly, the upper reference value for the fraction of hyperchromic RBC (2 %) and our inclusion criterion (10 %) are indicated as horizontal lines. The number of patients in each section of the plot is given

history of the selected patients was reviewed and patients were classified as discussed below (Fig. 2). An elevated proportion of hyperchromic RBC in patients with one or more known reasons for an acquired disease (Table 1) was considered acquired. If a hereditary membranopathy such as HS, explaining the elevated fraction of hyperchromic erythrocytes, had been diagnosed before, the patient was categorized as known congenital. All other patients were classified as having an unknown condition. Medical chart review revealed evidence for viral infections such as hepatitis B or C or human immunodeficiency virus (HIV) in many cases; therefore, we divided the unknown cases into two subgroups: Patients with and without documented viral infection. Cases of the latter group were excluded from further follow-up if the hyperchromic fraction had been within the normal range at least once in the past (not persistent). Even though an occasional presence of a normal fraction of hyperchromic erythrocytes in the patient's history does not exclude a congenital cause with certainty, it at least makes a transient or acquired cause more likely. For the remaining individuals (possibly congenital), a workup was done by determining the size of the spleen and performing a Coombs test and osmotic gradient ektacytometry on their RBC. For comparison and as internal controls, we tested several patients with a fraction of hyperchromic RBC increased to more than 10 % and known congenital hyperchromasia, documented viral infection, and acquired reasons for hyperchromasia.

Osmotic gradient ektacytometry

RBC deformability was studied with an ektacytometer (Technikon, Bayer, Leverkusen, Germany) in the osmoscan mode as described [10, 12–14]. Thereby, deformability of erythrocytes was measured by laser diffractometry. Deformability was plotted against the extracellular osmolality of a viscous solution of 20 % dextran that provides a constant shear stress at increasing osmolality. Dextran with an average molecular weight of 70 kDa was used (Carl Roth GmbH, Karlsruhe, Germany); this solution was buffered with 10 mM NaKHPO₄ to minimize the effect of pH on deformability [15] and also contained glucose (5.6 mM) and sodium azide (0.4 g/L). The osmolality gradient was obtained by adding sodium chloride to the solution in one compartment of the gradient mixer. The Technikon ektacytometer measures conductivity of the RBC suspension close to the diffractometer. The system was calibrated beforehand by measuring the ektacytometer's conductivity on a series of solutions differing in osmolality by cryoscopic osmometry (Gonotec Osmomat 030). An aliquot of 500 µl whole blood, collected in a standard 10-ml BD K2H Vacutainer® (18.0 mg of K₂EDTA), was mixed with 3 ml of isoosmolal dextran solution and directly inserted into the ektacytometer for measurement of RBC deformability. Blood samples were processed within 1–4 h after donation and incubated at room temperature prior to mixing with dextran. All samples were measured twice; both values consistently showed an almost

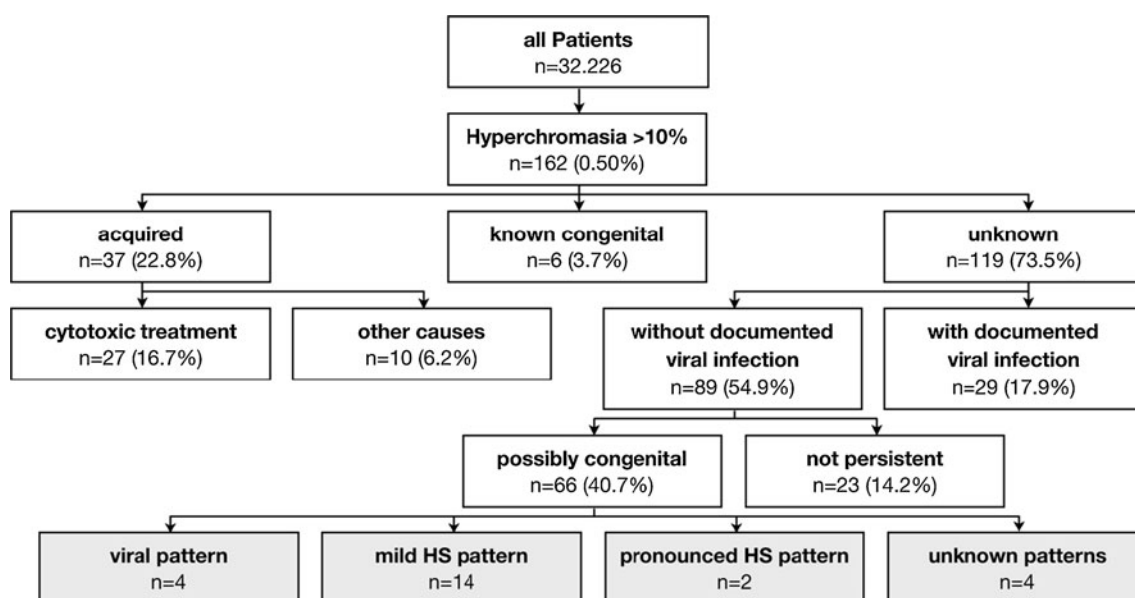


Fig. 2 Study design. During 6 months, all hematograms acquired were reviewed. Patient records were analyzed retrospectively if the hyperchromic RBC fraction was larger than 10 %. Patients with a known RBC disorder or a known condition leading to an elevation of hyperchromic RBC were thus classified as having a known congenital or an acquired hyperchromasia. All other patients (unknown) were separated

into patients with and without documented viral infection. Patients with nonpersistent elevation of hyperchromic RBCs were excluded from follow-up. The condition of the remaining patients was regarded as possibly congenital. Of these, 24 could be further classified by osmotic gradient ektacytometry

perfect correlation. The analog output of the Technikon ektacytometer was digitalized using a 12-bit A/D-Converter (NI USB-6008, National Instruments, Austin, TX, USA). On the digitalized osmoscan obtained, the following points were calculated: P_{\max} —the point of maximal deformability index (DI) and P_{\min} —the point of minimal DI (in the hypoosmolal branch). In addition, the osmolalities where half the DI of P_{\max} was reached on both the hypoosmolal branch (P'A) and the hyperosmolal branch (P'B) were recorded (Fig. 3a). In order to compare the results from patients and controls, we also recorded and analyzed osmoscans from 30 healthy volunteers. From these data, an averaged osmoscan profile was calculated. The respective range (double standard deviation) of the four points P_{\min} , P_{\max} , P'A, and P'B are indicated as boxes in the graphs (Fig. 3). These reference values were used for qualitative and quantitative evaluation of patients' samples.

Determination of P_{\min} , P_{\max} , P'A, and P'B for HS

To acquire discriminatory values from osmoscans of HS patients, we reviewed 15 osmoscans from patients with proven HS. Ten of these patients had a spectrin/ankyrin deficiency and five had a band 3 deficiency as verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [10]. The osmoscans had been obtained between 1999 and 2004 and were not digitalized but plotted onto paper. Therefore, the four characteristic points were determined manually from the plots.

Results

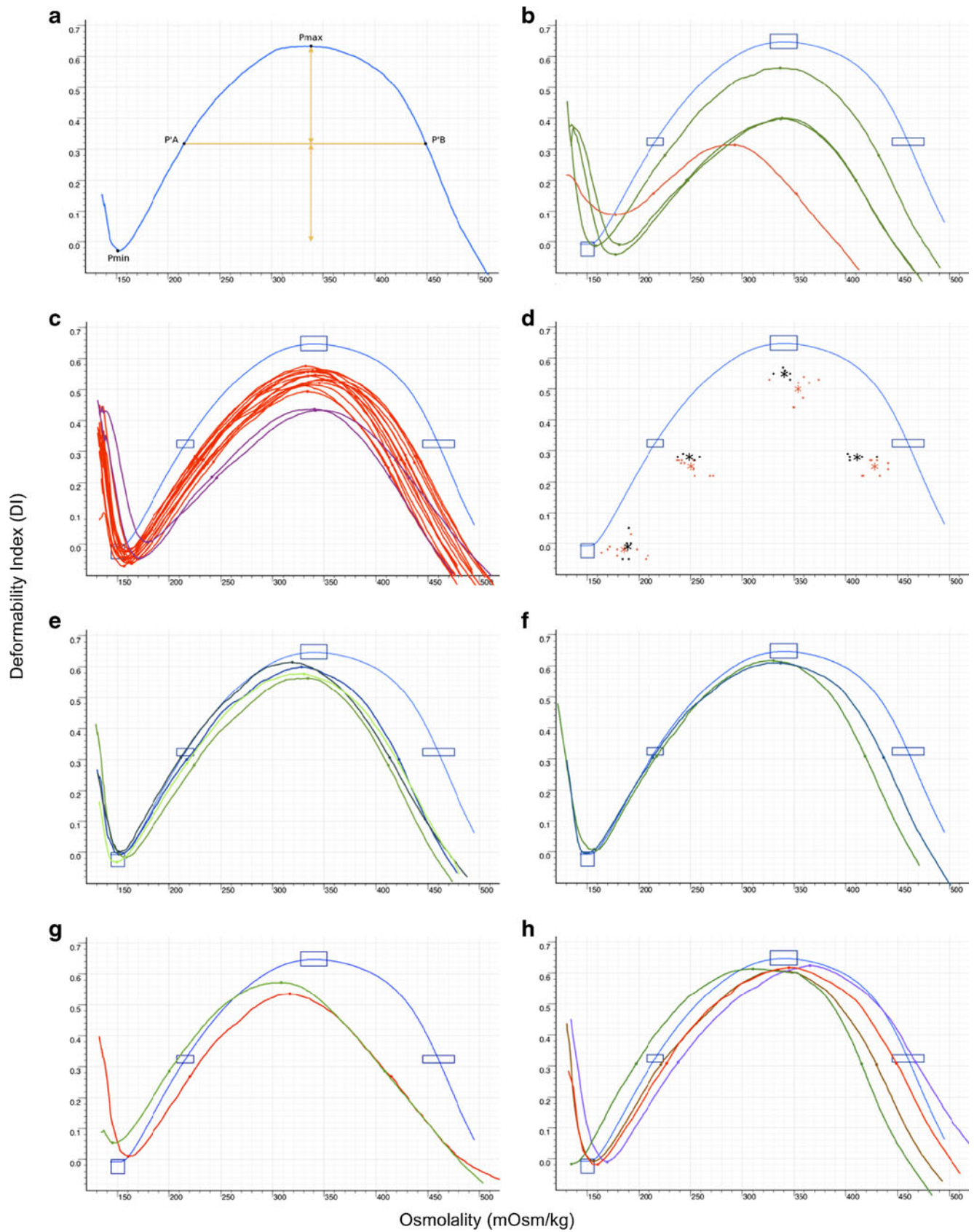
Comparing the percentage of hyperchromic RBCs with MCHC

We evaluated standard hematograms acquired during a period of 2 months at our institution. Thirty-eight thousand five hundred seventy-nine samples of 14,988 individuals were tested. Overall, values obtained for MCHC and the percentage of hyperchromic erythrocytes (Fig. 1) correlated strongly ($r=0.6$, $p<0.0001$, Spearman's rank correlation). Interestingly, this correlation was weaker at the extremes of both parameters (MCHC >360 g/l and percentage of hyperchromatic RBCs >10 %), confirming that MCHC and percentage of hyperchromatic RBCs cannot be used interchangeably. Of note, only 63 % of the 40 patients with more than 10 % or more hyperchromatic RBCs had an MCHC above the cutoff. Vice versa, only 14 % of the 250 patients with an MCHC >360 g/l had a percentage of hyperchromatic RBCs of 10 % or above.

Fig. 3 Osmoscans. **a** Characteristic points of osmoscans. P_{\min} is the point of 50 % hemolysis. P_{\max} is the point of maximal deformability. On the hyperosmolal and hypoosmolal branches of the osmoscan, the osmolality at which half the maximal deformability (half DI of P_{\max}) is reached are correspondingly defined as P'A and P'B. **b** Patients with a known hereditary membranopathy of the RBC. Three patients show different forms of HS (green), one patient has cryohydrocytosis (red). The ektacytometric features of HS consist of P_{\max} with deformability well below the normal range, though at a normal osmolality, and often an increased osmolality of P_{\min} , indicating a diminished osmotic resistance. The osmolality of P'A is increased, whereas that of P'B is shifted to hypoosmolal conditions. For comparison, we added the mean curve of 30 healthy donors (blue) into all the following osmoscans. The blue boxes indicate the normal range of the four characteristic points P_{\max} , P_{\min} , P'A, and P'B (double standard deviations). **c** Osmoscans of patients with possibly congenital elevation of the hyperchromic RBC fraction revealing a pattern of HS. Fourteen patients' osmoscans (red) reveal a mild form of HS, whereas 2 (purple) impress as more pronounced forms of HS. All osmoscans show the typical features of HS with a decreased maximal deformability and a loss of cellular solutes. The osmolality at which P_{\min} is reached is for the majority of cases only slightly higher than that of the controls. This implies a minute reduction of the osmotic resistance and probably explains why all of these patients were asymptomatic. **d** Osmoscans of HS patients with SDS-PAGE confirmed membrane protein deficiencies. The black points originate from five patients with band 3 deficiency, the red points from ten patients with spectrin/ankyrin deficiency. The averages of the characteristic points for the two types of disorders are marked by asterisks in the corresponding colors. **e** Osmoscans from patients with possibly congenital elevation of the hyperchromic RBC fraction revealing a viral pattern. P_{\min} and P'A are in the normal range, P_{\max} is slightly diminished and shifted to hypoosmolality, whereas P'B shows a clear shift to hypoosmolality. This solitary shift is characteristic for aged RBC having lost solutes prematurely. The normal osmolality of P_{\min} indicates a normal osmotic resistance of the RBC from these patients. **f** Osmoscans from two patients with documented viral infection. These osmoscans are very similar to those shown in Fig. 3e. **g** Osmoscans from two patients with acquired membranopathies. One patient was undergoing cytotoxic treatment (green curve) and one patient had AIHA, shown in red. **h** Osmoscans from four patients with possibly congenital elevation of the hyperchromic RBC fraction revealing unknown patterns. We could not assign these osmoscans to a specific RBC membranopathy, even though all curves suggest some sort of membrane disorder. The green curve fits to the pattern of a mild form of dehydrated stomatocytosis, with all characteristic points being shifted to hypoosmolality and the maximal deformability being only slightly diminished. The red curve possibly shows a borderline type of HS, with a slightly diminished osmotic fragility (P_{\min}) and a slightly diminished maximal deformability (P_{\max}). The brown curve shows a pattern reminiscent of a viral infection, except for the right shift of the left arm of the osmoscan

Analysis of patients with more than 10 % hyperchromic RBC

Hematograms from 32,226 individual patients were analyzed during a period of 6 months at the Division of Hematology of the University Hospital of Zurich. Out of these samples, 162 (0.50 %) contained more than 10 % hyperchromic RBC (Fig. 2). Review of patients' records revealed an acquired reason for this finding in 37 patients (22.8 %): An ongoing cytotoxic treatment was the most frequent



condition identified, explaining the elevated hyperchromic fraction of RBC in 27 patients; the 10 remaining patients summarized under the heading other causes included 3 with autoimmune hemolytic anemia (AIHA), 3 with severe plasma hypoosmolality, and 1 each with hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, an aortic valve defect, a massive myocardial infarction, and recent surgery for aortocoronary bypass. The group of patients with a known congenital explanation for the elevation of hyperchromic erythrocytes comprised six individuals (3.7 %) including five patients with HS and one with cryohydrocytosis [16], based on current and previous hematologic records at our hospital. For 119 patients (73.5 %), classified as unknown, we found no explanation for the elevated hyperchromic fraction. In these cases, we did not find evidence for any of the known reasons for hyperchromasia. Instead, 29 of these 119 patients were found to have acute or chronic viral infection. Most of these patients (22) were infected with HIV, 14 of them under documented antiretroviral therapy regimen. Some patients were infected with hepatitis C virus (seven, thereof three coinfections with HIV). Few patients suffered from chronic (two) or acute (one) hepatitis B virus infection.

For 23 of the remaining patients, hyperchromasia was not persistent; in these cases, we postulated an acquired cause. Finally, for a group of 66 patients, the elevation of the hyperchromic fraction was unexplained and likely to be persistent; this group would include patients with undiagnosed possibly congenital RBC membranopathies. Laboratory findings of the different groups are shown in Table 2.

Ektacytometry was performed on 32 subjects; 4 of these were of the known congenital group (3 HS and 1

cryohydrocytosis) and were aware of their condition. Their osmoscans are shown in Fig. 3b, illustrating a mild case of HS, two pronounced cases of HS, and one case of genetically confirmed cryohydrocytosis [16]. Sixteen of the remaining 28 patients examined by ektacytometry showed osmoscans characteristic for HS, with 14 displaying a mild form of HS and 2 displaying a more pronounced form of HS (Fig. 3c). None of these patients had been diagnosed with HS before.

Next, we compared the osmoscans of these newly diagnosed patients with the osmoscans of previously described HS patients [10] (Fig. 3d); the type of HS in the latter individuals had been determined by SDS-PAGE [10, 17], demonstrating band 3 deficiencies (five patients, black symbols) and spectrin/ankyrin deficiencies (ten patients, red symbols). Importantly, the osmoscans of both groups showed virtually overlapping characteristics: A significantly decreased maximal deformability and a lower surface to volume ratio, as indicated by a left shift of the hyperosmolal arm of the osmoscan. There is no doubt that the diagnosis for all patients whose osmoscans are shown in Fig. 3c, d is HS due to the characteristic osmoscan pattern.

In addition to HS, our analysis also revealed other characteristic osmoscan patterns; for example, a group of four patients with a possibly congenital elevation of the hyperchromic RBC fraction showed osmoscans in which the left arm was virtually identical to the controls, but the right arm was shifted to lower osmolalities (Fig. 3e). This pattern is highly similar to the osmoscans of two patients with documented viral infection, as shown in Fig. 3f. The underlying pathophysiological reasons of this osmoscan pattern are

Table 2 Basic characteristics of patient groups

Units (reference)		Acquired		Congenital	Unknown		
		Cytotoxic	Other		Not persistent	Possibly congenital	Viral
Number of patients		27	10	6	23	66	29
Sex	M/F	15:12	5:5	4:2	20:3	48:19	27:2
Age	Years	54 (18–90)	54 (20–89)	36 (20–44)	60 (25–104)	41 (21–84)	46 (26–73)
Hemoglobin	g/l (117–170)	115 (73–155)	117 (65–152)	148 (130–172)	153 (130–172)	143 (81–176)	144 (48–176)
Reticulocytes	% (0.40–2.50)	3.35 (0.00–10.42)	4.78 (1.7–15.16)	2.34 (1.50–3.94)	2.43 (0.57–5.64)	2.94 (0.94–12.30)	1.87 (0.48–4.43)
MCHC	g/l (310–360)	364 (275–383)	370 (348–391)	364 (283–394)	371 (360–383)	370 (267–398)	372 (356–384)
MCV	fl (80–100)	91.0 (82.6–101.0)	85.7 (80.6–92.8)	82.8 (76.7–85.9)	86.2 (79.0–98.4)	85.2 (25.3–98.4)	90.5 (39.6–101.1)
MCH	pg (26–34)	33.4 (29.9–38.4)	31.7 (29.6–34.9)	31.6 (28.7–33.1)	31.9 (29.5–36.1)	32.5 (27.5–68.7)	34.4 (29.3–43.8)
LDH	U/l (240–420)	477 (224–791)	1,120 (400–4,656)	449 (357–546)	531 (240–1,980)	427 (236–791)	486 (66–790)
Bilirubin	μmol/l (<21)	93 (7–819)	39 (6–109)	33 (27–39)	15 (4–31)	15 (5–36)	33 (9–80)
Neutrophils	10 ⁹ /l (1.4–8.0)	5.90 (0.90–20.30)	12.65 (2.10–47.80)	5.57 (3.00–8.80)	5.07 (1.20–19.10)	5.58 (0.50–46.30)	3.25 (1.20–6.70)
Lymphocytes	10 ⁹ /l (1.5–4.0)	1.02 (0.09–2.80)	6.70 (0.40–54.70)	2.36 (1.19–5.24)	1.37 (0.34–4.16)	2.53 (0.54–43.10)	1.82 (0.28–3.44)
Thrombocytes	10 ⁹ /l (143–400)	216 (35–471)	173 (34–310)	386 (196–851)	207 (71–366)	243 (32–547)	186 (28–366)

The mean and the range of the observed values are indicated

unclear; since such a pattern seems to accompany several viral diseases, we refer to it as a viral pattern.

Furthermore, the remaining four tested patients assigned to the possibly congenital group had unique osmoscans, with one fitting the diagnosis of dehydrated stomatocytosis [18] (Fig. 3h, green line). The remaining three could not be assigned to a specific disorder (Fig. 3h), pointing to as yet uncharacterized membranopathies or atypical presentations of known conditions. Interestingly, as shown in Fig. 3g, the osmoscans of two patients with acquired reasons for hyperchromasia including one patient with AIHA and one after cytotoxic therapy also showed a characteristic pattern not shared by any other group. Importantly, none of the patients with >10 % hyperchromic erythrocytes had a normal osmoscan pattern.

Discussion

In this study, we screened a large number of patients using the percentage of hyperchromic RBC and did a follow-up of patients with more than 10 % hyperchromic erythrocytes. Follow-up of these patients revealed a potentially relevant medical condition in the majority of these patients. Our data, therefore, argue for the clinical value of an elevated fraction of hyperchromic RBCs as a noteworthy parameter. If the percentage of hyperchromic RBCs is elevated to more than 10 % and an acquired condition for this shift can be excluded, further follow-up including tests for viral infections and hereditary membranopathies should be considered.

The majority of patients with more than 10 % hyperchromic erythrocytes also have an elevated MCHC; however, this correlation is not strict: More than a third shows an MCHC within the normal range. Vice versa, an elevation of the MCHC is also not strictly associated with a marked elevation of hyperchromic erythrocytes, indicating a certain independency of these parameters.

Among all patients undergoing a standard hematogram at our institution during a period of 6 months, 162 (0.5 %) were found with more than 10 % hyperchromic RBC. Sixty-six of these patients (41 %) remained as candidates for a

congenital but so far undiagnosed RBC membrane disorder. None of the 32 patients examined by ektacytometry revealed a normal pattern of RBC deformability. Sixteen thereof were found with osmoscans typical for HS (Fig. 3c); all of them without clinical symptoms. Osmoscans allowed the classification into a mild form of HS (14 patients) and a pronounced form (2 patients), in agreement with other clinical findings (Table 3). Comparisons of these osmoscans with those recorded from patients with known membrane protein deficiencies confirmed that all of these newly discovered patients have HS.

According to our data, the prevalence of HS is comparable to previously published data [19, 20] and may be higher than generally considered. Assuming that the patients tested by ektacytometry would be representative for the “possibly congenital” patient group, the number of HS-positive patients in this group can be extrapolated to be 44 (67 %). Adding the 5 patients with previously known HS, the estimated number of HS patients within the 162 patients with hyperchromasia greater than 10 % would be 49 (30 %). The prevalence of HS in our collective of 32,226 patients can be calculated to be at least 1:650. Since the sensitivity of our screening test “hyperchromasia >10 %” is not known, an unknown number of HS patients might have been missed by our study and the prevalence of HS would be even higher. However, it is not known whether the patient collective undergoing standard hematogram analysis at our institution is representative for the Swiss population.

Interestingly, we frequently identified viral infections in patients with more than 10 % hyperchromic RBC. In fact, of the 119 patients with neither a known congenital membranopathy nor a condition for an acquired hyperchromasia, 29 (24 %) had a documented viral infection, typically hepatitis or HIV, strongly indicating that these infections can cause hyperchromasia. To the best of our knowledge, this association has not been described previously. One explanation for our finding would be an accelerated clearance of older RBC following certain viral infections, a phenomenon which could account for a decrease of the right arm of the osmoscan inhibition [21, 22]. In agreement with this interpretation, the mean reticulocyte count was within the normal

Table 3 Characteristics of patients studied by ektacytometry

	Reference	Viral	Mild HS	Pronounced HS	Unknown
Number of patients		4	14	2	4
Spleen diameter	mm (<120)	130 (122–137)	140 (130–155)	136 (101–170)	136 (185–230)
Reticulocytes	% (0.4–2.5)	2.0 (1.2–2.5)	2.5 (1.8–4.4)	6.4 (3.8–9.0)	6.1 (2.6–12.3)
Hemoglobin	g/l (117–170)	154 (135–162)	152 (139–176)	108 (87–128)	136 (81–165)
LDH	U/l (240–420)	382 (314–449)	373 (343–398)	531 (492–570)	543 (308–778)
Bilirubin	μmol/l (<21)	22 (10–34)	14 (5–36)	30 (28–31)	14 (7–21)

The mean and the range of the observed values are indicated

range among the four cases studied by ektacytometry (Table 3). Whether such a premature clearance of older RBC is due to side effects of antiviral medication, which many of the patients with viral infections received, remains to be studied.

In our study, congenital RBC membranopathies, which are considered contraindications for blood donations by several institutions [23], were detected at a surprisingly high rate. Furthermore, some of the remaining patients suffered from viral infections including HIV. However, any potential benefit of such an additional test for the safety of a blood donation can only be demonstrated by a prospective study.

The osmoscans of RBC from healthy donors only show minute variations (Fig. 3), whereas those of RBC from patients with an acquired or hereditary hyperchromasia show a broad spectrum of variance, depending on the cause of hyperchromasia. Importantly, some osmoscan patterns could not be attributed to any of the known RBC membranopathies, potentially pointing to as yet undiscovered RBC abnormalities or disorders. The considerably elevated spleen diameters (Table 2) found in patients of this group support this hypothesis. These considerations clearly support a careful workup of these patients including acidified glycerol lysis test and eosin-5'-maleimide binding test [24].

Taken together, our study suggests an elevated fraction of hyperchromic RBC as a simple test for hyperchromasia to screen for hereditary RBC membrane disorders. Since this test identifies individuals with contraindications for blood transfusion, the use of this parameter could potentially improve the quality of blood donations.

Acknowledgments The ethical committee of the University Hospital of Zurich granted ethical approval for this study, which, therefore, has been conducted in accordance with the 1964 Declaration of Helsinki. All authors had access and full control of all primary data. If requested, the journal will be granted access to the primary data.

Conflicts of interest The authors declare no competing financial interests.

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